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Endothelial nitric oxide synthase polymorphism and prognosis in systolic heart failure patients

Naiel Azzam [a,b], Barak Zafrir [c,d], Fuad Fares [a,b], Yoav Smith [e], Nabeeh Salman [f,g], Roman Nevzorov [f,g], Offer Amir [f,g,*]

[a] Department of Human Biology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel
[b] Department of Molecular Genetics, Carmel Medical Center, Haifa, Israel
[c] Department of Cardiovascular Medicine, Lady Davis Carmel Medical Center, Haifa, Israel
[d] Ruth and Bruce Rappaport School of Medicine, Technion – Israel Institute of Technology, Haifa, Israel
[e] Genomic Data Analysis Unit, Hadassah Medical School, Hebrew University of Jerusalem, Israel
[f] Department of Cardiovascular Medicine, Poriya Medical Center, Tiberius, Israel
[g] Faculty of Medicine, Bar-Ilan University, Israel

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ABSTRACT

Background: The endothelial nitric oxide synthase (eNOS) gene single nucleotide polymorphism G894T is associated with thrombotic vascular diseases. However, its functional significance is controversial and data are scarce concerning its influence in heart failure (HF).
Methods: We studied 215 patients with chronic systolic HF. DNA was analyzed for eNOS gene G894T polymorphism using PCR and DNA sequencing. Evaluation of clinical characteristics and analysis of factors associated with 2-year mortality were performed for the homozygous G-allele G894T variant (GG), relative to the TT and GT variants.
Results: The genotype distributions of eNOS G894T alleles were: GG 135 patients (63%) and TT/GT 80 (37%). Two-year mortality was significantly higher in the GG variant (48%) than the combined TT/GT group (32%). The usage of nitrates was associated with increased 2-year mortality (HR 2.0, 95% CI 1.28–3.17; $p = 0.003$), which was most significant in the GG group treated with nitrates (73.5%) in comparison to the TT/GT group not treated with nitrates (34%); HR 2.75, 95% CI 1.57–4.79, $P < 0.001$.
Conclusions: Homozygosity for the G allele of the eNOS G894T polymorphism was associated with worse survival in systolic HF patients, especially in those treated with nitrates. ENOS polymorphism may result in different mechanistic interactions in HF than in thrombotic vascular diseases, suggesting that overexpression of NO may be associated with deleterious effects in systolic HF.

1. Introduction

Endothelial nitric oxide synthase (eNOS) is a key enzyme in the vascular endothelium, participating in the biosynthesis of nitric oxide (NO) from L-arginine in endothelial cells [1,2]. NO regulates changes in peripheral vascular tone, coronary blood flow and myocardial contractility, all important contributors in Heart failure (HF) disease. Moreover, inflammation and oxidative stress are also integral components in the progression of HF [3,4]. Accordingly, changes in eNOS activity and NO bioavailability may play an important role in the formation of reactive oxygen species and development of endothelial dysfunction, compromising cardiac performance and affecting HF progression [5].

The human eNOS gene is located on chromosome number 7. Several eNOS genomic polymorphisms have been described in association with pathological cardiovascular conditions; among them is the exon 7 G894T eNOS polymorphism, leading to amino acid substitution at position 298 (conversion of glutamate to aspartate) [6]. The clinical significance of the G894T eNOS polymorphism in humans was assessed in multiple studies, and significant association was found between this eNOS polymorphism and various cardiovascular conditions, including vasospastic angina, hypertension, and carotid and coronary atherosclerosis [7–14]. Several recent meta-analyses concluded that eNOS G894T polymorphism may increase the risk of developing thrombotic diseases and may play an important role in the development of hypertension, ischemic stroke and coronary heart disease [15–19]. Nevertheless, these studies were conducted in specific ethnic populations, and there is great divergence in research results. Moreover, several studies have found no evidence for an association between the G894T polymorphism and cardiovascular disease [20–25]. Whether the T allele

* Corresponding author. Cardiovascular Department, Poriya Medical Center, Tiberius, Israel. Fax: +972 99560390.
E-mail address: oamir@poria.health.gov.il (O. Amir).

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of the eNOS G894T polymorphism is associated with a reduction in the functionality of the eNOS enzyme is also an issue of controversy [26–31].

In addition, the data concerning the effect of the eNOS polymorphism on the presence and progression of HF are scant and conflicting, as two studies found an association between the eNOS G894T polymorphism variants TT and GT and non-ischemic, but not ischemic cardiomyopathy [32,33]. We as others have shown in fact the strong correlation between the G allele of the eNOS G894GT polymorphism and the presence of atrial fibrillation in systolic HF [34–37]. Furthermore, genetic heterogeneity may also have impact on the responsiveness to pharmacotherapy, and therefore is also a subject of research in HF [38,39].

Accordingly, the aim of current study was to explore the relation between increased nitrate effect derived by eNOS G894T genetic polymorphism and mortality in systolic HF patients.

2. Materials and methods

2.1. Study population

The study population included 215 patients with chronic systolic HF, who were followed at a tertiary HF outpatient center. Chronic systolic HF was defined as HF syndrome persisting for more than 3 months and left ventricular ejection fraction (LVEF) of less than 40%. All patients underwent full medical examination, echocardiography, twelve lead electrocardiography and baseline laboratory tests. The patients' clinical characteristics are presented in Table 1. Cardiovascular pharmacotherapy and baseline laboratory blood tests were documented in each patient (Table 2).

Blood samples were taken from each patient and their DNAs were analyzed for the presence of exon 7 G894T eNOS polymorphism. Patients were grouped on the basis of presence or absence of the Asp298 variant [Asp298 homozygotes (TT) and heterozygotes (GT) were combined and compared with Glu298 homozygotes (GG)].

Data concerning all-cause mortality during the 2-year follow-up period were gathered for each subject from patients' electronic files and health maintenance organizations. The study protocol was approved by the Institutional Review Board of Carmel Medical Center, in Haifa, Israel. All patients participating in the study signed informed consent.

Table 1
Patients' clinical characteristics, according to the prevalence of the G/T alleles.

Parameters	eNOS G894T polymorphism variants		P-value
	GG (n = 135)	GT and TT (n = 80)	
Age, mean ± SD	65.8 ± 13.1	62.6 ± 13.3	0.09
Gender (M), n (%)	107 (79.3)	71 (88.8)	0.2
BMI, mean ± SD	28.4 ± 5.3	28.5 ± 5.3	0.9
Systolic blood pressure, mean ± SD	114.8 ± 22.9	115 ± 20.6	0.9
Ischemic cardiomyopathy, n (%)	85 (63)	52 (65)	0.7
NYHA grade:			0.4
I, n (%)	7 (5.2)	3 (3.7)	
II, n (%)	51 (37.8)	31 (38.8)	
III, n (%)	41 (30.4)	31 (38.8)	
IV, n (%)	36 (26.7)	15 (18.7)	
Hypertension, n (%)	73 (54.1)	44 (40)	0.9
Diabetes mellitus, n (%)	56 (41.5)	33 (41.3)	0.9
LVEF, mean ± SD	24.1 ± 6.4	24.5 ± 7.2	0.6
LVEDD, cm, mean ± SD	6.3 ± 0.8	6.2 ± 0.8	0.3

BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic dimension.

Table 2
Laboratory blood tests and therapies, according to the prevalence of the G/T alleles.

Parameters	eNOS G894T polymorphism variants		P-value
	GT and TT (n = 80)	GG (n = 135)	
Anti-platelets, n (%)	60 (75)	104 (77)	0.9
ACEI/ARB, n (%)	71 (88.8)	123 (91.1)	0.5
Beta blocker, n (%)	74 (92.5)	128 (94.8)	0.4
Nitrates, n (%)	7 (8.8)	34 (25.2)	0.003
Diuretics, n (%)	76 (95)	126 (93.3)	0.3
Spironolactone, n (%)	25 (31.3)	36 (26.7)	0.5
Digoxin, n (%)	35 (43.8)	59 (43.7)	0.9
Statin, n (%)	59 (73.8)	112 (83)	0.1
Hemoglobin, gr/dl, mean ± SD	12.7 ± 1.6	12.3 ± 1.6	0.1
Creatinine, median (25th ;75th percentiles)	1.04 (0.9;1.4)	1.28 (1.0;1.6)	0.2
Urea, median (25th ;75th percentiles)	46 (32;63)	53 (38;80)	0.2
CCT, mean ± SD	70.6 ± 35.5	67.2 ± 34.8	0.5
Sodium, mEq , mean ± SD	139 ± 3.1	139 ± 3.9	0.7
ICD/CRT n (%)	38 (41)	63 (47)	0.1

ACEI/ARB, angiotensin converting enzyme inhibitors/angiotensin receptor blockers; CCT, creatinine clearance time; ICD, implantable cardioverter defibrillator; CRT, cardiac resynchronization therapy.

2.2. DNA analysis

Genomic DNA of each sample was extracted from whole blood leukocytes using the High Pure PCR Template Preparation Kit (Roche, Germany). Extracted DNA was stored at –20 °C. Substitution of the amino acid Asp for Glu298 was detected by amplification and sequencing of relevant segment. PCR was performed, using 500 ng of genomic DNA as a template, with the following primers: forward, 5′-GTG GTC ACG GAG ACC CAG CCA ATG AGG-3′; reverse, 5′-CCA GCG CAG GCC CAG GGC TGC AAA CCA-3′. The conditions used for PCR (Eppendorf Thermocycler, Hamburg, Germany) were as follows: (predenaturation) at 94 °C for 3 min, followed by 30 repeated cycles of denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min, extension of 45 s at 72 °C, and finally extension of 10 min at 72 °C. PCR products were analyzed by DNA sequencing using the sequencing services of Hylabs Laboratories, Israel.

2.3. Statistical analysis

The results are presented as the mean ± SD for continuous variables and as numbers and percentages for categorical data. Continuous variables with abnormal distribution are presented as interquartile range. T-test was used for comparison of the continuous variables or chi-square test for categorical data with the use of Fisher's exact test if needed. When the distribution was abnormal, the Mann–Whitney test was applied accordingly. For univariate and multivariable analysis of two-year survival Cox proportional hazards regression model was applied. The initial selection of the variables entered in the model was based on univariate analysis significance with inclusion criteria of $p < 0.10$. The results of the Cox proportional hazards model are presented as the hazard ratio (HR) with 95% confidence interval (CI). Kaplan–Meier method and comparison between groups of patients with the eNOS GG allele variant vs. TT/GT were performed by log-rank test. A two-sided p-value < 0.05 is considered as statistically significant. The statistical analysis was performed with SPSS software (version 16.0).

3. Results

The mean age of the study cohort was 65 ± 13 years and 83% were males. Ischemic cardiomyopathy was noted in 64%. Mean LVEF was 24 ± 7% and 57% were diagnosed as NYHA functional grade III–IV.